Cloning and Transformation of Hemagglutinin Cleavage Site Gene of Avian Influenza Virus Subtype H9N2 Into E.coli BL21

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Background & Objectives: Low pathogen avian influenza viruses including H9N2 subtype have been identified as an etiological infectious agent of serious economic loss in poultry industry in Asia, Middle East, Europe and America. In addition, due to the potency of H9N2 subtype to cause the next pandemic influenza in human, finding effective Methods for vaccination and diagnosis of the virus infection is important. Current study was conducted to clone and transform hemagglutinin cleavage site gene of H9N2 influenza A virus (strain A/Chicken/Iran/ 772/1998) into *E. coli* BL21.

Methods: The virus was grown in the allantoic cavities of 9-day, embryonated chickens' eggs and the genomic RNA was extracted. A set of primers which carried NheI and HindIII restriction cut sites, were designed to amplify a 585 bp fragment of besides the cleavage site of hemagglutinin (H9) gene. Then the amplified fragment and plasmid expression vectors including pET28a and pET26b were firstly digested with restriction enzymes and after ligation, transformed intoEcoli BL21 using calcium chloride. The clones were analyzed by PCR using specific and also universal T7 primers and sequencing.

Results: The target region was successfully cloned in the pET28a and pET26b vectors. PCR with both specific and universal T7 primers and also sequencing confirmed the cloned gene completion and its correct position.

Conclusion: Current research is a basic experiment for cloning and transformation of a 585 bp fragment of besides the cleavage site of hemagglutinin (H9) gene of AIV. Further research will be needed to express this fragment and evaluate the immunogenicity of this expressed HA protein in the lab animal models which challenged with influenza isolates.

Keywords: Cloning, Transformation; Hemagglutinin; Influenza